

POTENTIAL OF "ENERGY CROPS" OR MULTI-USE CROPS ON SALINE OR MARGINAL LAND

Year 3 Report

by

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SUMMARY

The overall objectives of this study were to identify high yielding crops for marginal and particularly saline soils, and then to develop methods for converting these into liquid fuels (ethanol) and coproducts such as feed.

A number of new lines of slender wheatgrass were evaluated on both saline and non-saline soils. Several new lines were found which gave significantly higher yields than the present licensed variety, Revenue.

In the crop screening phase of the project, fodder beets and kochia produced 13.4 and 11.7 t/ha, respectively on saline soil. Quinoa (preliminary trial) and kochia produced the highest yields, 15.5 and 15.0 t/ha, respectively, on non-saline land. Jerusalem artichoke yielded 19.6 t/ha under irrigation on sandy soil.

Considerable progress in processing forage crops was made. The yield of sugars obtained during enzymatic hydrolysis of polysaccharides from forages was dependent on maturity. More sugars per unit weight of forage were released from mature than from young samples. However, the percent of cellulose hydrolysis was decreased with increased forage maturity. Soil salinity decreased the yield of sugars.

None of the pretreatments to increase the ease of cellulose hydrolysis were found to be equally effective for all forage species, mainly because of the differences in forage composition. Pin milling was the most promising physical pretreatment. While there was no increase in the yield of sugars, the requirement for cellulolytic enzymes was decreased by one-half. Ammonia freeze explosion significantly increased the enzyme reactivity of some forages.

The yields of alcohol obtained during fermentation of alfalfa and Jerusalem artichoke were comparable to values reported in the literature. The distillers solids contained high protein contents and lysine was 84 to 91% available. The solids can be fed directly to animals in an adjacent feedlot or dried for storage.

Enzymatic proteins were concentrated and purified by ultrafiltration and diafiltration at constant volumes. With this simple method, 89-94% of enzyme activity was recovered. Similar yields of reducing sugars were obtained during hydrolysis of forage with recovered and fresh enzymes. However, lower glucose yields suggest that β -glucosidase activity was lost and that supplementation with fresh enzyme was necessary.

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1. INTRODUCTION

Background

Two million hectares of cultivated land in Saskatchewan are affected by high soil salinity, of which 400,000 hectares are sufficiently saline to severely affect crop production (Holm and Henry, 1982). Many saline spots are scattered throughout fields otherwise not affected by salinity problems. Secondly, there are significant areas along the northern and eastern fringes of agricultural land in western Canada where the frost hazard is substantial and the growing season is too short to permit regular harvest of cereals or

oilseeds. There are also large areas on the prairies which are affected by drought.

The objective of the SRC program was to investigate the potential for high yielding salt-tolerant or drought-tolerant crops adapted to growing on such marginal lands. The initial results on yield and feed value of these crops could have immediate application in cattle production. Forages and other crops which can be grown on poor quality or saline lands are also poten-

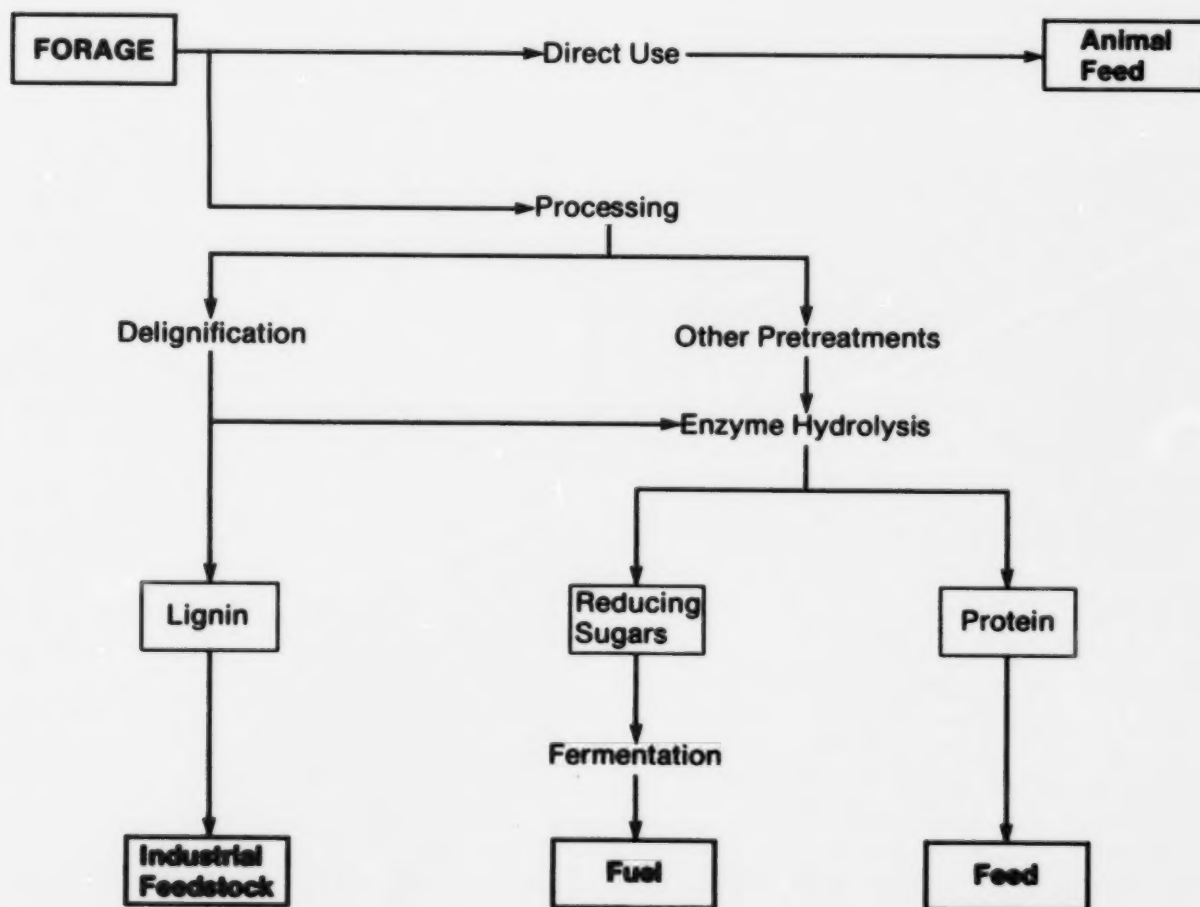


Figure 1. Utilization of forage components.

tial sources of polysaccharides, which can be fermented to liquid fuel after conversion to simple sugars. One of the major problems which has deterred the commercialization of the latter process is that forages are not uniform substrates. The ratio of cellulose:hemicellulose:lignin in forages varies from species to species, and also changes with maturity. Most of the cellulose is in an unreactive form, and lignin-hemicellulose bonds must be broken before free carbohydrates are formed (Morrison, 1974). Therefore, the objectives of pretreatment were to increase the reactivity of cellulose, to break bonds between lignin and hemicellulose, and to increase the available surfaces for hydrolysis by the means of physical or chemical action. Once cellulose and hemicellulose are made accessible and reactive by pretreatments, cellulase and hemicellulase enzymes can be used to hydrolyze cellulose and hemicellulose to simple sugars. These sugars can then be fermented to ethanol by yeasts.

Fungal cellulases, e.g. from *Trichoderma reesei*, are composed of several constituents, including endo- and exo- β -1,4-glucanases and β -glucosidases (Mandels, 1982). The hydrolysis of cellulose proceeds through the synergistic action among these components. However, with increasing conversion of cellulose to glucose, the hydrolysis rate will decrease due to increasing resistance of the residual substrate, product inhibition and enzyme inactivation (Reese and Mandels, 1980). Enzymes are considered to be the major expense (40-60%) in the process of saccharification of cellulose (Jogleker et al., 1982). Enzymes were reported to be second (20-32%) only to the cost of raw materials in the total cost of alcohol production, (Moreira et al., 1981).

Improvement in the economics of biofuel production could be achieved by utilization of all forage components, e.g., lignin as an industrial feedstock and protein as animal feed (Figure 1), and by recycling enzymes.

While energy (liquid fuels) is the primary focus ('Energy Crops'), utilization of the coproducts is very important to the overall economics, hence the alternative designation of "Multi-Use Crops".

Objectives

- 1) To find crops, particularly forages, which are high yielding on saline land.
- 2) To find crops which produce well on other types of marginal land.
- 3) To investigate the potential of selected salt tolerant crops and crops for other marginal land, as sources of carbohydrates for fermentation and by-product protein for animal feed.

For forage crops studied:

- 3.1) To investigate the influence of physical or chemical pretreatments on the yield of simple, fermentable sugars from the enzyme hydrolysis of forages.
- 3.2) To recover enzyme proteins after the hydrolysis process and to investigate the feasibility of their re-use.
- 3.3) To economically recover protein fractions after enzyme hydrolysis and fermentation, and to investigate the nutritional quality of these protein fractions.

The realization of Objectives No. 1 and 2 could bring immediate economical benefits by increased utilization of marginal land and production of less expensive animal feed.

The development of an economical method of biomass refining to produce liquid fuel has a long-term potential. This potential is influenced by availability and price of conventional energy sources, the price of enzymes and the cost of processing forages.

SOIL SALINITY OF SASKATCHEWAN

Legend

Salinity Hazard

Very low to low

Moderate

High to very high

% of quarter sections with salinity deductions

1-25

26-50

51-100

0 20 40 60 80 100

MILES

KILOMETERS

after L.S. Crosson,
Saskatchewan Institute of Pedology
University of Saskatchewan
Publication No. M29, 1976

Legend

Salinity
Hazard
Very low to low

Very low to low

Moderate

High to very high

% of quarter
sections with
salinity
deductions

1-25

5-50

-100

1

after L. S. Crosson,
Saskatchewan Institute of Pedology
University of Saskatchewan
Publication No. M29, 1976

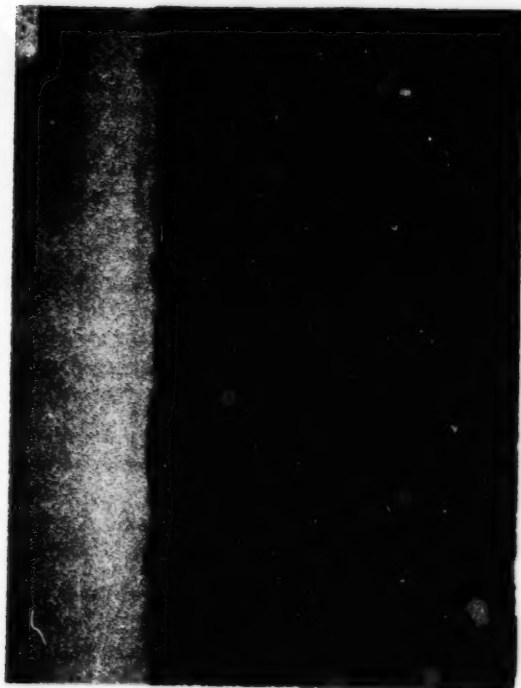


Plate 1. Sunflower. Yield on dry land at mid-dough stage was 11,000 kg/ha

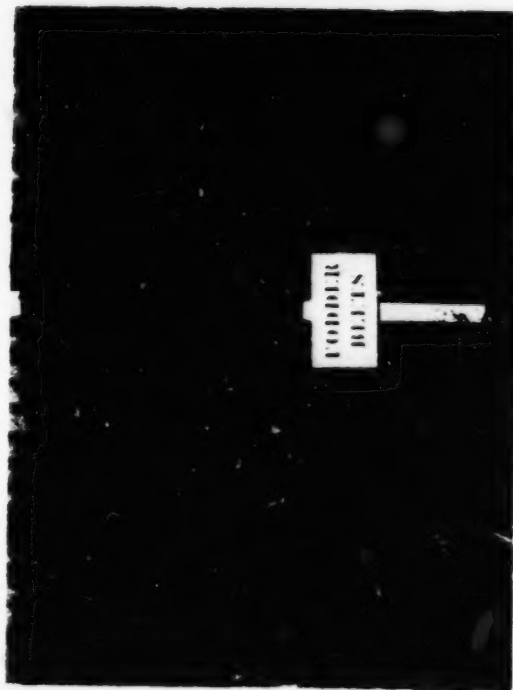


Plate 3. Fodder beets. Yield on saline soil, at maturity stage, was 13,448 kg/ha

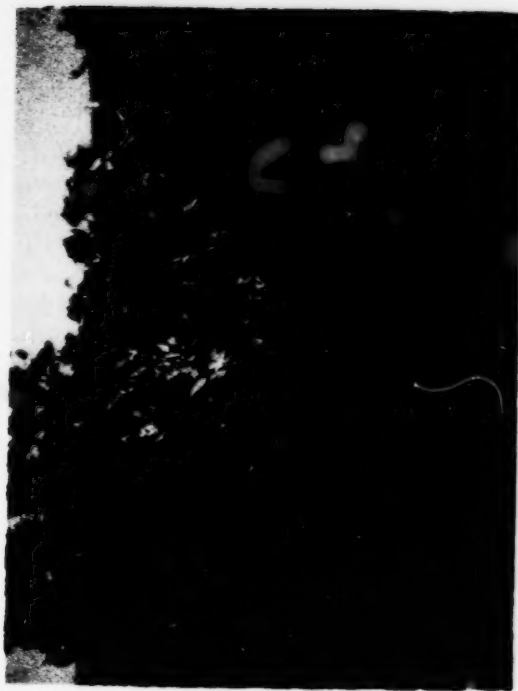


Plate 2. Quinoa. Yield on dry land at flower stage was 15,487 kg/ha



Plate 4. Jerusalem Artichoke. Yield under the irrigation at maturity stage was 19,636 kg/ha (Tubers plus crop residue)



Plate 5. Kochia. The yield on saline soil, at flower stage, was 11,666 kg/ha



Alfalfa



Ryegrass

Plates 6 and 7. Crops grown in Saskatchewan as part of the agronomic screening program

2. MATERIALS AND METHODS

Agronomic Studies

Twelve slender wheatgrass (SWG) genotypes, the licensed SWG (Revenue), tall wheatgrass (Orbit), crested wheatgrass (Summit) and wild ryegrass (Altai) were grown on a saline gradient near Langham, Saskatchewan, during 1981-1982. The six replicate randomized block experiment was situated on a slope starting from the edge of a slough. The unusual crops for Saskatchewan, Jerusalem artichok (JA) and fodder beets, were grown on saline seep near Swift Current. Two common weeds, milkweed¹ and kochia², and barley, a typical Saskatchewan crop, were also grown on the same site. Kochia was also grown on a saline site near Webb, Saskatchewan.

The same perennial grasses and seven common crops: sunflower (Sundak and Corona), triticale (Carman), mustard (Domo), barley (Bonanza), fababeans (Hertz Freya), canola (Regent), wheat (Neepawa) and oats (Cascade) were grown on dry land at Saskatoon. Five unusual crops: oilseed radish (Apollo), ryegrass (Lirisant), a SWG-wheat cross, flat-pea and quinoa (Puno-15, Puno-2, Mario-tapin and Mbric-tapier) were also grown on dry land at Saskatoon. JA was grown on sandy soil under irrigation.

The total plant material was harvested from each plot at anthesis and at maturity and analyzed for crude protein (CP), phosphorus, organic matter and organic matter digestibility as a measure of their nutritional value for cattle feed. The yield of digestible organic matter and CP per ha were calculated. The salinity of the soil on the saline gradient was determined by electrical conductivity (EC) (paste method) and ion analysis.

¹*Asclepias speciosa* Torr.

²*Kochia scoparia* L. Schrad

Laboratory Experiments

Three forages were selected for laboratory experiments: kochia, SWG and alfalfa (control). The influence of forage maturity and soil salinity on the yield of fermentable sugars obtained from enzyme hydrolysis was investigated. To improve the yield of fermentable sugars the following pretreatments were studied: pin-milling, homogenization and the ammonia freeze-explosion process (AFEX) (Dale and Moreira, 1982), as well as extraction in alkali, acid, alcohol and acid-alcohol mixture. Forages were also treated with ultrasound in the presence of acid, alkali and enzymes.

The feasibility of extracting protein from fresh and dried forages, followed by enzymatic hydrolysis of extracted residues, was investigated. The proteins were also recovered from several fractions after enzyme hydrolysis or/and fermentation. However, the major effort was placed on the recovery of the most valuable and expensive proteins, enzymes, by ultrafiltration and diafiltration at constant volume. Recovered and purified enzymes were re-used to hydrolyse other forage fractions. All results are reported on a dry matter basis unless otherwise indicated.

Fundamental Studies

The structure of forage cell walls and the "mechanism" of treatment to improve the yield of fermentable sugars after enzyme hydrolysis were discussed in the Year 3 report on "Fundamental Chemistry and Biology of Forages and Crop Residues". Observed differences between mono- and dicotyledons were discussed. Finally, literature reviews on the microscopic studies of cell wall structure and on the effect of plant phenolics on cellulolytic microorganisms was presented in that report.

3. RESULTS

Crop Yields

Crops grown on dry land in Saskatchewan were harvested at 3 stages of growth: anthesis, mid-dough and maturity. The highest yields were obtained at the mid-dough stage (Table 1). Two crops, sunflower and oats, produced 11,000 kg/ha of forage, while the average of all other crops was 9,000 kg/ha.

At maturity, the yield of all crops declined due to loss of leaves (Table 1). The differences in yields between mid-dough and mature stages were only 5% for triticale, but 37% for canola. The seeds accounted for 26-47% of the total plant materials harvested at maturity.

The highest yields for most of the investigated crops (sunflower, oats, triticale, canola, fababean, wheat and mustard) were obtained at their mid-dough stage (Table 2). Quinoa, in preliminary trial, milkweed, and the perennial grasses, gave the highest yield at flower and at anthesis stage of their growth. Three crops, kochia, flat-pea and JA, gave the highest yields at maturity.

Very high yield on dry land was obtained for quinoa at the flowering stage* and kochia at the mature stage (Table 2). Other crops gave only one quarter to two-thirds the yields of quinoa and kochia. Sunflower and oats, harvested at mid-dough stage, yielded about 2,000 kg/ha more plant material than triticale, canola, fababeans, wheat, mustard and flat-pea. SWG and SWG-wheat cross gave their highest yields (7914 and 6719 kg/ha respectively) when they were harvested at anthesis stage. The lowest yields among dry land crops were obtained for ryegrass and milkweed. JA gave a low yield on dry land but a very high yield (19,636 kg/ha) under irrigation.

Fodder beets and kochia gave the exceptionally high yields among the crops grown on a saline site, where the salinity was in the range of 11-12 mS/cm (Table 3). Barley gave a yield of 5,600 kg/ha at EC 15 mS/cm. JA did not tolerate a high salinity of 11.6 mS/cm and gave a very low yield.

For SWG trials, the genotype 1269 was the most salt tolerant (up to 9 mS/cm). Other perennial grasses yielded well at EC values below 6 mS/cm (mild salinity).

* Quinoa would not set seed, possibly due to the long hours of daylight in Saskatchewan.

The yield of these crops grown on saline soils compared favorably with the yield of grain or forage obtained on cultivated land. Alfalfa yields on non-saline soils in the vicinity of these plots were 4140 to 5320 kg/ha (two cuts). Barley grain yields on non-saline soils were 4040 kg/ha.

Sugar Yield

The amount of sugars determined after acid hydrolysis of the fiber fraction was influenced by species, maturity of sample and growth conditions (Table 4). The sugars in alfalfa and kochia accounted for 34.4-42.5 and 28.3-41.0% of DM, respectively. Both the sugar and the glucose levels increased with maturity (Table 4).

The opposite was observed for SWG forage as the levels of sugars and glucose declined with maturity. Also, salinity of soil had a deteriorating influence on the glucose level (Table 4), based on a limited number of samples.

More sugars per unit weight of forage were released from mature than from young samples by enzyme hydrolysis (Table 5). However, the percent of cellulose hydrolysis decreased with increasing forage maturity.

Pretreatments

None of the pretreatments were found to be equally effective for all forage species, mainly because of the differences in forage composition. The degree of lignification, protein contents, presence of phenolics, tannins and saponins and other enzyme inhibitors have been reported by others to influence the success of pretreatment and sugars yield. The highest yield of hydrolysed cellulose was obtained after alkali extraction of alfalfa (81.3%) (Table 6).

Extraction of kochia with 95% ethanol, 0.02N sulfuric acid and sonication in hemicellulase solution produced almost equal conversion of cellulose to simple sugars (Table 6). The AFEX pretreatment was the most effective for SWG. Almost 90% of cellulose was hydrolysed to fermentable sugars.

Pin-milling was found to be the most promising among the physical pretreatments (Table 6). Samples of forage were ground to 400 mesh or finer on an Alpine Pin Mill. There was no wet processing involved,



Flat-pea



Fababean



Milkweed

Plates 8, 9, 10. Crops being evaluated in the agronomic screening program

no chemicals were added and therefore there was no need for recovery of chemicals, washing of the residues and disposal of wastes. The yields of cellulose hydrolysed to glucose were lower than those obtained by optimum pretreatments for particular forages. However, as compared to other treatments, the requirement for enzymes to hydrolyse the pin-milled forage was reduced to one-half, due to increased surface area. This further decreased the cost of saccharification.

More drastic methods must be employed to improve the sugars yield during saccharification. Among those pretreatments which should be tested are steam-explosion process (Rowe et al. 1982), AFEX (Dale and Moreira, 1982) or a new process involving high pressure carbon dioxide (Puri, 1983). The economic feasibility, safety and equipment requirements will determine which of these pretreatments is the most efficient. Our results suggest that a combination of increased surface area, and increased cellulose reactivity is required.

Fermentation

During preliminary fermentations, JA forage gave the highest yield of reducing sugar (RS) after enzyme hydrolysis (Figure 2), and yielded 11.2% ethanol per kg of dry forage (Table 7). This represented an alcohol conversion of 53.5%.

Initial trials with untreated kochia and alfalfa gave low yields of ethanol when enzyme hydrolyzed forages were fermented with *Saccharomyces* yeast. Preliminary experiments with *Kluyveromyces marxianus* in this procedure gave higher yields, suggesting that further trials with this micro-organism and treated forages would be worthwhile. The yield of ethanol obtained from untreated alfalfa using *K. marxianus* is similar to that reported by Chua-Yap and Dale (1982). It should be emphasized that only initial trials with fermentations have been done, and much remains to be studied in selection of pretreatments, enzyme hydrolysis, and fermentation methods.

Enzyme Recovery

An important aspect of the process of the biofuel production was cost and recovery of enzymes. Certain pretreatments decreased the enzyme requirements drastically. Enzymes were recovered by con-

centration and purification of the liquid fraction at constant volume on a Pellicon Cassette Ultrafiltration Unit. The membrane cut point was 10,000 MW. During the process of enzyme recovery 89% of the enzyme activity was recovered and 97% of RS were separated from the concentrated solution (Table 8, Figure 2).

In one experiment, losses of enzyme activity were limited to about 6%, as compared to enzyme activity before the saccharification process. The recovery of enzymes was probably influenced by the presence of enzyme inhibitors or compounds which precipitate protein such as saponins or tannins. Their level may depend on the type of pretreatment and the composition of the forage. Further studies would be required to confirm these hypotheses.

Enzymes were also recovered after the fermentation process. Almost 20% of the activity was lost during saccharification and fermentation of kochia and alfalfa. No activity was lost following fermentation of JA (Table 9). The enzymes could also be recovered after a second and third saccharification process although activity loss increased with each re-use.

Enzyme Re-use

After hydrolysis of fresh forage with recovered enzymes, a similar yield of total RS was obtained compared to samples hydrolysed with fresh enzymes (Table 10). However, lower yields of glucose obtained with recovered enzymes compared to fresh enzymes, suggest the need for supplementation of lost β -glucosidase activity (Table 10).

Protein Recovery

Although "enzymes" are the most valuable part of "biomass protein" (enzyme, forage and yeast proteins present after enzyme hydrolysis and/or fermentation), the other proteins should also be recovered and utilized to lower the overall cost of biofuel production.

Separation of the liquid fraction removed 58-68% of total biomass protein (Table 11, Figure 2). This fraction contains enzyme protein recoverable by ultrafiltration, and solubilized and hydrolysed forage protein. The latter are only 15% recoverable by standard acid precipitation (Table 11). These hydrolysed proteins plus hemicellulose sugars could be concentrated by heating to obtain molasses for ruminant

feed.

The residue contained 31-42% of the total biomass protein of which very little could be recovered as a pure protein fraction (Table 11). The residue after enzyme hydrolysis and/or fermentation contained part of the forage protein and a portion of the enzyme protein absorbed on the residue. Yeast protein was

also present in distillers solids. The protein content of distillers solids was similar to or higher than the original forage, and the lysine availability was about 90% (Table 12). Thus, distillers solids (residues after enzyme hydrolysis) could be used as animal feed without any further processing, or after drying.

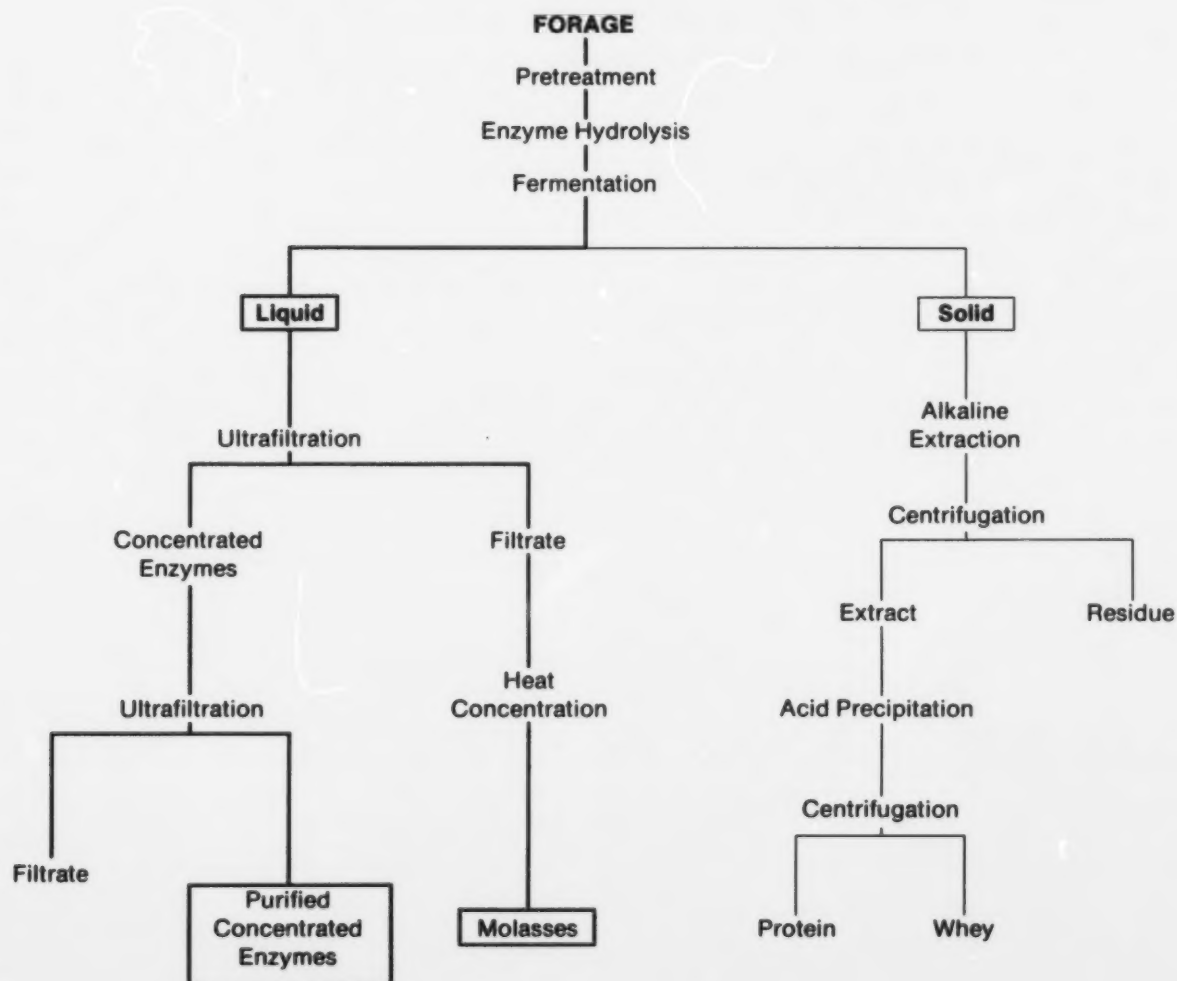


Figure 2. Feasible ways of recovery of proteins after enzyme hydrolysis and fermentation.

4. CONCLUSIONS

1. New high yielding forages, such as kochia, have been identified, and may make suitable cattle feed. Several new lines of SWG were identified with higher yield potentials than Revenue.
2. Fodder beets and kochia gave the highest yields on saline soil.
3. Quinoa and kochia produced the highest yields, 15.5 and 15.0 t/ha, respectively, on dry land.
4. More sugars were released per unit weight of mature than young forage, however, the percentage of hydrolysed cellulose decreased.
5. None of the evaluated pretreatments were found to be equally effective for all forage species, mainly because of the differences in forage composition.
6. Pin-milling was the most promising physical pretreatment. The enzyme requirement was decreased by one-half. AFEX treatment was very effective in increasing the extent of enzyme hydrolysis of cellulose in SWG forage.
7. Over 90% of enzyme activity was recovered by ultrafiltration and diafiltration.
8. Similar yields of RS were obtained during hydrolysis of forages with fresh and recovered enzymes. However, low glucose yields were obtained with recovered enzymes, suggesting lost β -glucosidase activity.
9. High protein content, and lysine being 90% available made the distillers solids good quality animal feed.

5. RECOMMENDATIONS

1. Further field trials should be carried out to develop kochia and new lines of SWG as forages for saline soils.
2. The dicotyledon forages, e.g. kochia and alfalfa, had sufficiently low concentrations of cellulose and hemicellulose that enrichment techniques such as leaf-stem separation should be tested prior to enzyme hydrolysis.
3. Further studies on pretreatments should be conducted to establish the best conditions to improve the reactivity of cellulose and hemicellulose to hydrolyzing enzymes. A combination of increased surface area and chemical treatment appears desirable.
4. Once the best pretreatment and enzyme hydrolysis conditions are established, fermentation experiments to maximize the yield of ethanol should be conducted.

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TABLE 1
YIELD AT THREE STAGES OF DEVELOPMENT OF VARIOUS SASKATCHEWAN CROPS
GROWN ON DRY LAND AT SASKATOON DURING 1962.

Crop	Cultivar	Yield At	Yield At	Yield at Maturity Stage		Vegetative Fraction
		Anthesis Stage	Mid-Dough Stage	Whole Plant	Seed	
		kg/ha				
Sunflower	Corona	9010	11460	8760	3920	4840
Oats	Cascade	7390	10960	8350	3940	4410
Barley	Bonanza	5620	9900	7840	3300	4510
Canola	Regent	4910	9290	5810	1520	4290
Triticale	Carman	5790	9280	8730	3200	5530
Fababean	Hertz Freya	3440	9130	7630	3470	4160
Wheat	Neepawa	5750	9090	7780	2990	4790
Mustard	Domo	6680	8550	7020	2370	4650

TABLE 2
MAXIMUM DRY MATTER YIELDS, PRODUCTION OF DIGESTIBLE MATTER AND CRUDE PROTEIN YIELDS
OBTAINED ON DRY LAND OR IRRIGATION (OBTAINED FROM WHOLE PLANTS)

Crop	Cultivar or Genotype	Stage of Maturity	Dry Matter Yield	DOM*	Protein Yield
		kg/ha			
Dry Land					
Quinoa	Puno-15	Flower	15,487	6,600	2,741
Kochia	Scoparia	Mature	15,010	6,553	1,501
Sunflower	Corona	Mid-dough	11,459	5,933	985
Oats	Cascade	Mid-dough	10,965	5,766	998
Triticale	Carman	Mid-dough	9,287	4,819	724
Canola	Regent	Mid-dough	9,294	4,835	1,348
Fababean	Hertz Freya	Mid-dough	9,135	5,722	1,452
Wheat	Neepawa	Mid-dough	9,089	4,606	636
Mustard	Domo	Mid-dough	8,991	4,466	1,412
Fiat-Pea	<i>L. tingitanus</i>	Mature	8,859	4,077	585
SWG	1307	Anthesis	7,914	3,556	649
SWG-wheat	hybrid	Anthesis	6,719	3,804	880
JA	—	Mature	5,768	4,261	295
Ryegrass	Bentra	Anthesis	4,854	2,742	748
Milkweed	—	Anthesis	3,630	2,434	428
Irrigation					
JA	—	Mature	19,636	13,877	1,739

*DOM = Digestible Organic Matter

TABLE 3
MAXIMUM YIELDS AND PRODUCTION OF DIGESTIBLE ORGANIC MATTER AND PROTEIN
OBTAINED FROM WHOLE PLANTS GROWN ON SALINE SOIL.

Crop	Cultivar (Genotype)	Stage of Maturity	Yield	DOM	Protein Yield	Soil Salinity mS/cm
			kg/ha			
Fodder Beets*	—	Mature	13,448	9,093	1,264	11.2
Kochia	Scoparia	Flower	11,666	5,381	1,167	12.1
Barley	Bonanza	Mature	5,612	3,131	505	15.0
SWG	1302	Anthesis	4,330	1,965	407	6.6
	1269	Anthesis	2,750	1,442	303	9.1
Jerusalem Artichoke	—	Mature	1,960	1,426	190	11.6

*Under the irrigation the yield of tubers was 30600 kg/ha.

TABLE 4
THE INFLUENCE OF MATURITY ON THE COMPOSITION OF CARBOHYDRATES,
DETERMINED AFTER ACID HYDROLYSIS OF FORAGES.

Forage	Stage of Maturity	Carbohydrate Content (%)						
		Arabinose	Xylose	Fructose	Galactose	Glucose	Mannose	Total
Alfalfa								
	Prebud	1.41	3.16	1.47	1.62	25.60	1.17	34.43
	10% Bloom	2.56	10.96	—	1.13	27.13	0.76	42.54
Kochia								
	Pre-bud	2.98	4.16	0.70	1.16	18.39	0.95	28.34
	Pre-flower	2.53	6.82	1.00	1.21	20.86	1.44	33.86
	Flower	3.64	8.68	0.84	1.79	21.59	1.59	38.13
	Post Flower	3.86	8.29	—	2.04	22.77	1.65	38.61
	Mature	3.72	9.67	—	1.65	24.32	1.65	41.01
SWG								
	Anthesis	1.33	19.50	0.90	0.95	39.64	tr.	64.70
	Anthesis*	2.38	19.06	0.95	0.92	34.46	0.92	58.69
	Mature	2.33	13.43	0.65	0.91	33.14	9.88	51.34

*saline site.

TABLE 5
THE INFLUENCE OF FORAGE MATURITY AND SOIL SALINITY ON THE YIELDS OF HYDROLYSED DM, FIBER AND CELLULOSE.

Forage	Hydrolysed DM ¹ %	Hydrolysed Fiber ² %	Hydrolysed Cellulose ³ %
Stage of Maturity			
Alfalfa			
Bud	17.1	50.5	65.2
10% Bloom	24.6	57.8	68.0
Kochia			
Pre-bud	24.1	73.2	81.5
Pre-flower	18.4	52.6	57.8
Flower	17.4	45.4	56.0
Post Flower	18.8	46.0	53.0
Mature	18.3	39.2	47.8
SWG			
Anthesis	28.8	44.6	56.8
Anthesis (saline site)	21.7	36.5	42.5
Mature	26.1	39.8	54.1

¹Hydrolysed DM = RS as % of DM

²Hydrolysed Fiber = RS as % of Fiber

³Hydrolysed Cellulose = Glucose as % of Cellulose

RS = Reducing Sugars

TABLE 6
YIELDS OF HYDROLYSED CELLULOSE* AFTER ALTERNATE PRETREATMENTS.

Pretreatment	Forage	Glucose Yield (%)		
		Alfalfa	Kochia	SWG
No Pretreatment		43.5	22.3	25.6
Autoclaving		60.5	44.1	47.5
Fine Milling (pin-mill)		69.6	47.1	61.3
Coarse Milling (Wiley-mill)		41.7	36.8	40.0
Homogenization		45.6	24.2	31.2
Ultrasound Treatment: 0.05N H ₂ SO ₄		41.5	40.0	33.1
5% H ₂ SO ₄		52.0	51.2	21.2
0.05N NaOH		55.6	49.3	53.9
1% Hemicellulase		—	56.8	28.9
1% Pectinase		—	41.4	24.6
0.1% Trypsin		—	39.4	24.7
AFEX		71.7	—	89.2
Extraction: 0.1% NaOH		81.3	41.5	—
0.02N H ₂ SO ₄		48.9	56.7	41.0
0.02N H ₂ SO ₄ -butanol (11:5:1)		67.0	43.2	34.9
80% Ethanol		44.3	48.6	39.4
95% Ethanol		38.6	57.1	36.3
NDF Solution		50.7	32.8	30.6

*Hydrolysed Cellulose = Glucose as % of Cellulose.

TABLE 7
YIELD OF REDUCING SUGARS OBTAINED BY ENZYME HYDROLYSIS AND ETHANOL YIELDS FROM FERMENTATION OF FORAGES.

Forage	Stage of Maturity	RS ¹ After Enzyme Hydrolysis % Fiber	Yeast Strain ²	Ethanol Yield L/10 ³ kg Dry Forage	Ethanol Conversion ⁴ %	Maximum Ethanol Conversion %
JA	Bud	73.15	<i>S. cerevisiae</i>	111.7	53.5	64.0
Kochia	Flower	33.78	<i>S. cerevisiae</i>	44.3	21.3	64.0
Alfalfa	Bud	43.94	<i>S. cerevisiae</i>	30.5	16.0	64.0
Alfalfa	Flower	56.25	<i>K. marxianus</i>	101.2	53.1	64.0

¹Enzyme hydrolysis was carried on for 24 h.

²24 h culture

³Fermentation proceeded at 32°C for 24 h.

⁴Percent of maximum theoretical conversion of the cellulose.

TABLE 8
RECOVERY OF ENZYMES AFTER SACCHARIFICATION PROCESS.

	RS			Activity		
	mg/ml	Total mg	Removed %	IU/ml	Total IU	Recovered %
Filtrate	2.42	1977	—	2.33	1906	—
Concentrate of Enzymes	1.02	133	93.0	11.20	1456	76.4
Purified Enzymes	0.45	58	97.0	13.10	1703	89.0

TABLE 9
RECOVERY OF ENZYMES AFTER SACCHARIFICATION (S) AND FERMENTATION (F).

	Alfalfa Activity		Kochia Activity		JA Activity	
	Total IU	Loss %	Total IU	Loss %	Total IU	Loss %
Before S+F	997.0	—	997.0	—	997.0	—
After S+F	775.0	22.3	768.0	22.9	997.0	—
Recovered	815.0	18.3	799.0	19.9	1113.0	+11.6
After 2nd S	532.0	46.6	747.0	25.1	846.0	15.1

TABLE 10
COMPARISON OF PERFORMANCE OF RECOVERED AND NEW ENZYMES.

Forage	Recycled Enzymes				New Enzymes			
	RS % DM	GLC Sugars % DM	Glucose % Cellulose	RS % Fiber	RS % DM	GLC Sugars % DM	Glucose % Cellulose	RS % Fiber
Alfalfa	22.47	11.03	34.74	26.14	20.60	14.10	41.50	33.03
Kochia	20.80	15.40	44.16	39.29	22.03	19.49	56.32	49.73
JA	23.04	18.01	13.11	49.47	20.44	19.93	33.84	54.75

TABLE 11
PROTEIN DISTRIBUTION AND RECOVERY AFTER ENZYME HYDROLYSIS AND FERMENTATION OF ALFALFA.

	Protein			Protein Yield (% of Biomass Protein)			
	g/100 g	Distribution Liquid %	Residue %	Extracted	Precipitated	Whey	Extracted Residue
Forage	27.2						
AFEX	32.4						
Enzyme Hydrolysis							
— Forage Biomass	34.1	67.8	31.2	14.6	1.5	57.9	26.1
— AFEX Biomass	38.6	58.0	42.0	12.5	2.5	54.8	24.7
Fermentation							
— Forage Biomass	52.2	32.5	57.5	—	—	—	—

TABLE 12
NUTRITIONAL QUALITY OF FORAGES AND RESIDUES AFTER ENZYME HYDROLYSIS (ALFALFA ONLY)
AND ENZYME HYDROLYSIS AND FERMENTATION.

	Protein %	Total Amino Acids %	Lysine Total %	Available % of Total
Alfalfa				
— Forage	27.2	76.6	4.2	92.7
— Residue after Enzyme Hydrolysis	38.6	86.6	5.6	87.3
— Residue after Fermentation	32.2	69.5	4.0	90.3
Kochia				
— Forage	8.8	68.1	4.2	89.7
— Residue after Fermentation	7.2	76.4	4.5	84.5
JA				
— Forage	7.8	75.0	6.0	90.9
— Residue after Fermentation	14.1	80.1	6.4	90.3